PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#37

In re Application of
Jack A. ROTH *et al.*

Serial No. 08/233,002

Filed: April 25, 1994

For: METHODS AND COMPOSITIONS COMPRISING DNA DAMAGING AGENTS AND p53

Group Art Unit: 1804

Examiner: A. Milne

Atty. Dkt.: INGN:008/HYL

CERTIFICATE OF MAILING
37 C.F.R. 1.8

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4/17/96
Date

Steven L. Highlander

DECLARATION OF DR. JACK A. ROTH UNDER 37 C.F.R. §1.132

Hon. Asst. Commissioner
for Patents
Washington, D.C. 20231

I, Jack A. Roth, declare that:

1. I am a U.S. citizen residing at 2324 Bolsover, Houston, Texas. I am chairman of the Department of Cardiac and Thoracic Surgery at M.D. Anderson Cancer. A copy of my *curriculum vitae*, outlining my education and research training, are attached.

2. I am the Jack A. Roth named as an inventor on the above-captioned application. I also own an interest in INTROGEN THERAPEUTICS, Inc., which has licensed the above-captioned application.

3. It is my understanding that the examiner in charge of this application has taken the position that claims drawn to a combined therapy involving DNA damaging agents and transgenic p53 expression would not be believed to have therapeutic efficacy by those of skill in the art.

4. The specification contains several examples that demonstrate the efficacy of a combined therapy approach with respect to cancer therapy. Example 4 describes p53-directed expression from an adenoviral vector in tumor cells *in vitro* and inhibition of the tumor cells' growth thereby. Example 5 shows inhibition of tumor cell growth by and Ad-p53 construct in tumor cells *in vivo*. Example 6 shows inhibition of tumor formation and growth *in vivo* by Ad-p53 in a murine orthotopic lung cancer model. Examples 7 and 8 show the synergistic results observed when the Ad-p53 therapy is combined with cisplatin treatment. Taken together, these data show that the combination of p53 gene therapy and treatment with a DNA-damaging agent provide improved results with regard to tumor cell growth inhibition and tumor cell killing.

5. Two manuscripts relating to combined therapy have been prepared by myself and my colleagues. These manuscripts further demonstrate the operability of the present invention.

6. In "Adenoviral-mediated Wild-Type p53 Gene Expression Sensitizes Colorectal Cancer Cells to Ionizing Radiation," Spitz *et al.* (attached), adenoviral-mediated transfer of p53 into colorectal carcinoma cells treated with ionizing radiation show improved results in terms of tumor growth when compared to either the Ad-p53 or radiation treatments alone. Analysis of cells by TUNEL assay indicated increased apoptosis in treated cells. These results indicate that the tumor cell population not killed outright by Ad-p53 treatment alone is more sensitive to ionizing radiation. These results were observed both in *in vitro* assays and in *in vivo* studies using a murine subcutaneous tumor model.

7. In "Novel Gene Therapy Strategy for Human Non-Small Cell Lung Cancer: Combination of Sequential Cisplatin Administration and Adenovirus-mediated p53 Gene Transfer," Nguyen *et al.* (attached), prior treatment of tumor cells with cisplatin increased the inhibition of tumor cell growth when compared with Ad-p53 or cisplatin alone. TUNEL analysis indicated early onset of apoptosis in cells receiving both treatments. These observations were consistent between *in vitro* studies and *in vivo* experiments using a murine subcutaneous tumor model.

8. Therefore, it is my opinion that those of skill in the art would, in fact, read the instant specification and the data provided in the attached manuscripts as indicative of therapeutic efficacy for the claimed invention.

9. I hereby declare that all statements made herein of my knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under §1001 of Title 18 of the U.S. Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

5/13/96
Date

Jack A. Roth, M.D.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Jack A. ROTH *et al.*

Serial No. 08/233,002

Filed: April 25, 1994

For: METHODS AND COMPOSITIONS COMPRISING DNA DAMAGING AGENTS AND p53

Group Art Unit: 1806

Examiner: A. Milne

Atty. Dkt.: INGN:008/HYL

CERTIFICATE OF MAILING
37 C.F.R. 1.8

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December 18, 1996
Date

Steven L. Highlander

DECLARATION OF DR. JACK A. ROTH UNDER 37 C.F.R. §1.132

Hon. Asst. Commissioner
for Patents and Trademarks
Washington, D. C. 20231

I, Jack A. Roth, M.D., declare that:

1. I am a U.S. citizen residing at 2324 Bolsover, Houston, Texas. I am chairman of the Department of Thoracic and Cardiovascular Surgery at M. D. Anderson Cancer Center. A copy of my *curriculum vitae*, outlining my education and research training, are already of record.

2. I am the Jack A. Roth named as an inventor on the above-captioned application. I also own an interest in INTROGEN THERAPEUTICS, Inc., which has licensed the above-captioned application.

3. It is my understanding that the Examiner has questioned the enablement of claims drawn to methods of contacting a cell with a p53 protein or gene and a DNA damaging agent in a combined amount effective to kill said cell. More specifically, the Examiner has asserted that the specification is insufficient to enable successful killing of cells *in vivo* in humans.

4. Recently, a phase I clinical trial has been initiated for combined adenovirus-p53 (Adp53) gene therapy and cisplatin treatment of lung cancer patients. There are two treatment arms: 1) Patients receiving only adenovirus-mediated p53 gene therapy or; 2) patients receiving a combination of adenovirus-mediated p53 gene therapy and cisplatin. To date, 16 patients have been enrolled in the trial. The data from the first group of six patients, which received Adp53 at 10^6 plaque forming units (PFU)/dose, with or without cisplatin, is summarized below.

5. Patient 1 is a 45 year-old female, diagnosed with unresectable squamous cell carcinoma of the lung (T4N2M0) 27 months prior to entry into the protocol. For two months, the patient was treated with four courses of Vinblastin, followed by 63 Gy of radiotherapy to the lung for another three months. In September, 1995, the patient was

evaluated and found to have a right upper lobe mass. The patient was enrolled in the p53 gene replacement protocol in October, 1995. She received 4 cycles of one injection monthly of Adp53 at a dose of 1 x 10^6 PFU into the right upper lobe mass. The patient's response to Adp53 injection was graded as stable for the first two cycles and progression for the last two cycles. The patient was removed from the study on March 1,1996.

6. Patient 2 was a 60 year-old male, diagnosed with unresectable squamous cell carcinoma (T3N2M0) 5 months prior to study entry into the protocol. The patient had a history of squamous cell carcinoma of the larynx followed by a laryngectomy in February, 1994. Radiation therapy to the left and right upper neck of 5940 CGy and right lower neck of 6480 CGy was received between March, 1994 and April, 1994. The patient was treated with multiple courses of cisplatin, Mitomycin and Velban between July 1995 and August, 1995. The patient's primary tumor was in the left upper lobe of the lung; other sites including the left lower lobe, mediastinal lymph nodes and right hilar lymph nodes were also involved. The patient entered the clinical trial for p53 gene replacement on November 22, 1995. The patient received one injection of Adp53 of 1 x 10^6 PFU monthly for two months and then was removed from the study due to progression at the study site diagnosed via CT scan on January 9, 1996. The patient died 31 days later due to disease progression.

7. Patient 3 was a 75 year-old female, diagnosed with adenocarcinoma (T3N2M0) 11 months prior to entry into the protocol. The patient had a history of breast

cancer with a right breast mastectomy followed by 23 Cobalt treatments in 1964. Between March 1995, and January 1996, the patient was treated with multiple courses of chemotherapy including Navelbine, Taxol, Platinol and Carboplatin. The patient entered the protocol with a right upper lobe bronchus adenocarcinoma (study disease site) and right paratracheal subcarina adenopathy. The patient was treated with a single injection of Adp53 at a dose of 1×10^6 PFU on February 27, 1996. One month later the patient was removed from the study due to progression at the study disease site and died two and half months after study treatment due to disease progression.

8. Patient 4 is a 70 year-old female, diagnosed with adenocarcinoma (T2N2M0) four years prior to entry into the protocol. The patient had a history of breast cancer with infiltrating ductal carcinoma of the left breast in 1990 which was treated with a mastectomy in October, 1990. The patient did not receive chemotherapy. She developed carcinoma of the left lung in 1992, and received 60 Gy of radiation therapy. The patient entered the protocol with a left hilar mass, designated as the study disease site, and a right lower lobe adenocarcinoma. The patient received cisplatin at a dose of 80 mg/m^2 three days prior to a single injection of Adp53 at a dose of 1×10^6 PFU monthly for 6 months. The patient's disease was stable until September, 1996, at which time the patient was discontinued from the study due to progression.

9. Patient 5 is a 72 year-old male who was diagnosed with adenocarcinoma (T2N3M0) 28 months prior to entry onto the protocol. The patient was treated with

surgery to remove the left upper lobe and underwent bronchoplasty to reconstruct the mainstem bronchus in April, 1995. The patient had not been treated with chemotherapy or radiation therapy prior to study entry. The patient entered the study with a suprasternal mass at the disease study site, and also had involvement of the brain, a left axillary lymphadenopathy, left and right pleural effusions, and liver metastasis. The patient was treated with cisplatin at a dose of 80 mg/m^2 three days prior to a single injection of a Adp53 at a dose of 1×10^6 PFU. The patient received six courses of treatment with stable disease at the indicator site. The patient was discontinued from the study because of progression at other sites.

10. Patient 6 is a 45 year-old male who was diagnosed with adenocarcinoma (T4N2M0) 10 months prior to entry onto the protocol The patient was treated for nine months with multiple courses of cisplatin, Mitomycin, Velban, Taxol and Carboplatin. The patient did not receive any radiation therapy. The patient then presented with a right adrenal mass and also had involvement of the brain and left hilar lymph nodes. He entered the protocol on April 12, 1996. The patient received cisplatin at a dose of 80 m^2m three days prior to a single injection of a Adp53 at a dose of 1×10^6 PFU to the right adrenal mass. The patient received six courses of treatment with stable disease at the indicator site. He was discontinued from the study because of progression at other sites.

11. Based on the foregoing results, I believe that treatment of cancer patients with a combination of adenovirus-mediated p53 gene therapy and cisplatin shows efficacy at preventing tumor progression as compared to patients receiving p53 gene therapy alone.

12. I hereby declare that all statements made herein of my knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the U.S. Code, and that such willful false statements my jeopardize the validity of the application or any patent issued thereon.

12/13/96
Date

Jack A. Roth, M.D.